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Constituents from the roots of *Taxus cuspidata*

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Abstract The known propelargonidin, afzelechin-(4α→8)-afzelechin (**1**), the known lignans 7′-hydroxynortrachelogenin (**2**), epinortrachelogenin (**3**), nortrachelogenin (**4**), hydroxymatairesinol (**5**), allohydroxymatairesinol (**6**), matairesinol (**7**), oxomatairesinol (**8**), and isotaxiresinol (**9**), and the known taxoids taxinine M (**10**), taxayuntin (**11**), and 10-deacetyltaxol (**12**), and 10-deacetylbaccatin III (**13**) were isolated from the roots of *Taxus cuspidata* (Japanese yew, Taxaceae). The propelargonidin was isolated from *Taxus* spp. for the first time, and was detected in the roots, bark, and twigs.

Key words *Taxus cuspidata* · Roots · Propelargonidin · Lignan · Taxoid

Introduction

Taxus cuspidata Sieb. et Zucc. (Japanese yew, Taxaceae) is widely distributed in Japan, where it has been used as a garden tree and a folk medicine.[1] The plants of the genus *Taxus* are evergreen gymnosperms, with eight species existing, and are rich sources of biologically active diterpenoids belonging to the taxoids.[2–4] Phenolic compounds from *Taxus* spp. also show biological activity. The needles of *T. baccata* Linn. contain a phenylbutanoid glycoside rhododendrin, which shows hepatoprotective activity.[5] The aqueous extracts from the needles of *T. baccata* show tranquilizing and sedative activity, presumably related to the benzodiazepine-like activity of biflavones of the amentoflavone type.[6] The isolation of matairesinol, hydroxymatairesinol, isotaxiresinol, α-conidendrin, and secoisolariciresinol from *Taxus* spp. has been reported,[7–9] and these lignans are known as biologically active compounds.[10] Hydroxymatairesinol and matairesinol exhibit antifungal activity against *Fomes annosus* and *Lentinus lepideus*, respectively.[11,12] Shen et al.[9] confirmed the antitumor activities of isotaxiresinol, α-conidendrin, and secoisolariciresinol.

In this article, we reported the isolation and identification of a known propelargonidin, eight known lignans, and four known taxoids from the roots of *T. cuspidata* (Fig. 1).

Results and discussion

Compounds **1**–**13** were isolated from the roots of *Taxus cuspidata* by chromatographic methods. By comparing the spectroscopic properties, the known propelargonidin was identified as afzelechin-(4α→8)-afzelechin (**1**);[13] the known lignans were identified as 7′-hydroxynortrachelogenin (**2**), epinortrachelogenin (**3**), nortrachelogenin (**4**), hydroxymatairesinol (**5**), allohydroxymatairesinol (**6**), matairesinol (**7**), oxomatairesinol (**8**), and isotaxiresinol (**9**);[8,9,14–19] and the known taxoids were identified as taxinine M (**10**), taxayuntin (**11**), 10-deacetyltaxol (**12**), and 10-deacetylbaccatin III (**13**).[20–23] The known propelargonidin **1** and seven known lignans **2**–**8** and were isolated for the first time from *T. cuspidata*. Furthermore **1** has not been isolated from any other *Taxus* spp., while the isolation of **9**–**13** from *T. cuspidata* has been reported.[24,25]

The needles of *Taxus* spp. contain dimeric flavonoids of the amentoflavone type.[26–28] However, the propelargonidins had been never isolated from *Taxus* spp. The content of afzelechin-(4α→8)-afzelechin (**1**) in the various parts of a *T. cuspidata* tree were quantitatively analyzed by high-performance liquid chromatography (HPLC) (Table 1). The content of **1** was higher in the roots and the bark than in the other plant parts of a *T. cuspidata* tree; however, it was not detected in the wood and the needles.

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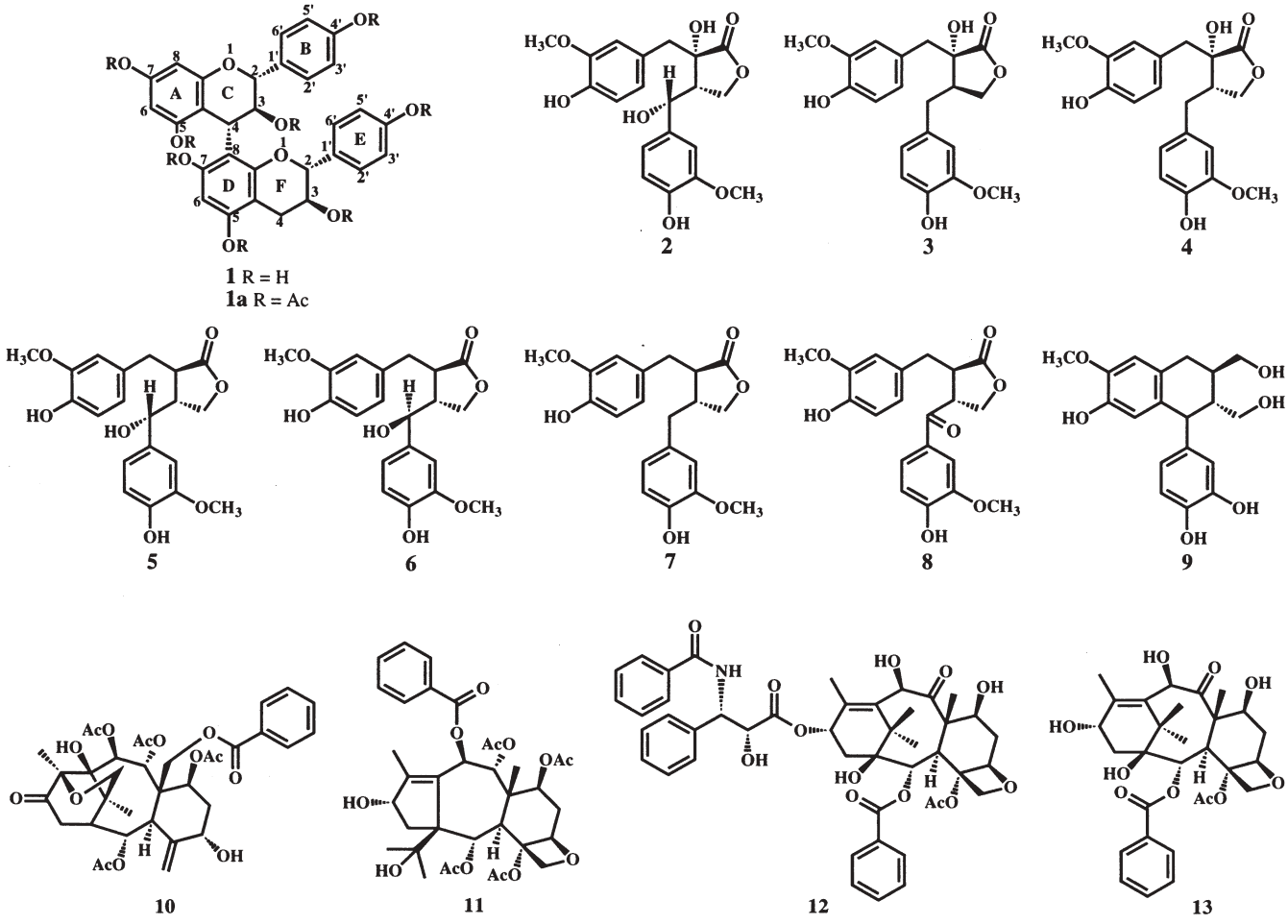

Fig. 1. Chemical structures of compounds **1**–**13**

Table 1. Content of afzelechin-(4α→8)-afzelechin (**1**) in various parts of *Taxus cuspidata*

Plant parts	Extracts (%)[a]	Content of **1** (%)[a]
Roots	13.17	10.6
Wood	6.41	–[b]
Bark	29.70	11.8
Twigs	17.55	5.6
Needles	52.56	–[b]

[a] Percentages based on oven-dried matter
[b] Not determined with reliability under the established analysis conditions

Materials and methods

General

^{1}H and ^{13}C NMR spectra were obtained with a JEOL JNM-LA400 (400 MHz) spectrometer. Optical rotations were determined with a JASCO DIP-140 polarimeter. The remaining analytical equipment, plant materials, and extraction and fractionation techniques were the same as in our previous report.[29]

Isolation

The air-dried root of *Taxus cuspidata* was extracted with methanol (MeOH)–dichloromethane (CH_2Cl_2)(1:1, v/v) at room temperature. The MeOH–CH_2Cl_2 extract (85.00 g) was extracted successively with *n*-hexane and ethyl acetate (EtOAc), and the EtOAc (38.09 g) solubles were obtained. A portion (10.00 g) of the EtOAc solubles was subjected to silica gel column chromatography (benzene:EtOAc, 9:1–0:10, v/v) and 95 fractions were collected in 100-ml portions. Eluates (9–21) were subjected to preparative HPLC (P-HPLC) (flow rate: 15.0 ml/min, detection: UV 280 nm, eluent: MeOH–H_2O, 50:50, v/v) to elute fractions (Fr.) 9-21-1–Fr. 9-21-11. Fraction 9-21-1 (8.2 mg) was resubjected to P-HPLC (MeOH–H_2O, 26:74, v/v) to afford the colorless amorphous 7′-hydroxynortrachelogenin (**2**)(2.0 mg) ($[\alpha]_D^{25}$ − 26.7° (*c* 0.17, MeOH), M^+ 390). (−)-Hydroxymatairesinol (**5**)(23.7 mg) ($[\alpha]_D^{25}$ − 11.6° [*c* 1.0, tetrahydrofuran (THF)], M^+ 374), (−)-allohydroxymatairesinol (**6**)(4.2 mg) ($[\alpha]_D^{25}$ − 10.0° (*c* 0.4, THF), M^+ 374), (−)-epinortrachelogenin (**3**)(7.6 mg) ($[\alpha]_D^{25}$ − 10.7° (*c* 1.00, MeOH), M^+ 374), and (−)-matairesinol (**7**)(30.6 mg) ($[\alpha]_D^{25}$ − 50.6° (*c* 0.75, MeOH), M^+ 358) were obtained from Frs. 9-21-2, 9-21-3,

9-21-5, and 9-21-7, respectively. Fraction 9-21-4 (6.5 mg) was resubjected to P-HPLC (MeOH–H_2O, 36:64, v/v) to obtain colorless amorphous (−)-nortrachelogenin (**4**)(2.4 mg) ($[\alpha]_D^{25}$ − 37.5° (*c* 0.20, EtOH), M^+ 374) and (+)-oxomatairesinol (**8**)(3.1 mg) ($[\alpha]_D^{25}$ + 12.0° (*c* 0.25, MeOH), M^+ 372). Eluates 32–42 were subjected to P-HPLC (MeOH–CH_3CN–H_2O, 35:30:35, v/v/v) to elute Fr. 32-42-1–Fr. 32-42-5. Fraction 32-42-3 (33.4 mg) was resubjected to P-HPLC (MeOH–H_2O, 65:35, v/v) to obtain colorless amorphous (−)-taxinine M (**10**)(20.2 mg) ($[\alpha]_D^{25}$ − 31.4° (*c* 1.31, $CHCl_3$)) and (−)-taxayuntin (**11**)(5.3 mg) ($[\alpha]_D^{25}$ − 50.7° (*c* 0.61, $CHCl_3$)) as colorless needles. (−)-10-Deacetyltaxol (**12**)(40.7 mg) ($[\alpha]_D^{25}$ − 41.6° (*c* 1.00, MeOH)) was obtained from Fr. 32-42-4. Eluates (43–50) were subjected to P-HPLC (MeOH–H_2O,45:55, v/v) to elute Fr. 43-50-1–Fr. 43-50-5. Fraction 43-50-1 (99.3 mg) was resubjected to P-HPLC (MeOH–H_2O, 34:66, v/v) to elute Fr. 43-50-1-1–Fr. 43-50-1-9. Fraction 43-50-1-1 (31.7 mg) was resubjected to P-HPLC (MeOH–H_2O, 21:79, v/v) to afford pale yellow amorphous (−)-afzelechin-(4α→8)-afzelechin (**1**)(1.8 mg) ($[\alpha]_D^{25}$ − 63.3° (*c* 0.15, MeOH)). Fraction 43-50-1-6 (6.0 mg) was resubjected to P-HPLC (MeOH–H_2O, 24:76, v/v) to afford colorless amorphous (+)-isotaxiresinol (**9**)(4.8 mg) ($[\alpha]_D^{25}$ + 34.3° (*c* 0.50, MeOH), M^+ 346). (−)-10-Deacetylbaccatin III (**13**)(102.0 mg) ($[\alpha]_D^{25}$ − 35.5° (*c* 0.59, MeOH)) was obtained from Fr. 43-50-5.

Quantitative analysis of afzelechin-(4α→8)-afzelechin (**1**)

A *T. cuspidata* tree was divided into roots, wood, bark, twigs, and needles, and the tree parts were extracted with MeOH–CH_2Cl_2 (1:1, v/v) at room temperature. The identification of HPLC peaks for afzelechin-(4α→8)-afzelechin (**1**) and quantitative determination by HPLC were based on co-HPLC analyses and calibration curves prepared with **1** isolated from the roots of *T. cuspidata*. The eluent and conditions for the analyses of **1** were as follows: eluent, MeOH–H_2O (18:82, v/v); flow rate, 0.5 ml/min; detection, UV at 280 nm; column oven temperature, 40°C. The retention time of **1** was 15.1 min.

Afzelechin-(4α→8)-afzelechin (**1**)

In the past reports of afzelechin-(4α→8)-afzelechin (**1**), the ^{1}H NMR spectral data for aromatic protons were not shown.[13,30] Colorless amorphous powder, $[\alpha]_D^{25}$ − 63.3° (MeOH; *c* 0.15). UV λ_{max}^{MeOH} nm (log ε): 274.4 (3.88), 225.2 (*sh*)(4.53), and 212.2 (*sh*)(4.64). ^{1}H NMR (acetone-d_6–D_2O): δ 2.53 [1H, *dd*, *J* = 16.2, 8.5 Hz, H-4α(F)], 2.94 [1H, *dd*, *J* = 16.2, 5.6 Hz, H-4β(F)], 3.75 [1H, *m*, H-3(F)], 4.34 [1H, *d*, *J* = 9.3 Hz, H-2(C)], 4.41 [1H, *m*, H-3(C)], 4.45 [1H, *d*, *J* = 7.3 Hz, H-4(C)], 4.59 [1H, *d*, *J* = 8.1 Hz, H-2(F)], 5.86, 5.95 [2H, each *d*, *J* = 2.4 Hz, H-6(A), H-8(A)], 6.21 [1H, *s*, H-6(D)], 6.69 [2H, *d*, *J* = 8.5 Hz, H-2′(E), 6′(E)], 6.77 [2H, *d*, *J* = 8.5 Hz, H-3′(B), 5′(B)], 6.92 [2H, *d*, *J* = 8.5 Hz, H-2′(B), 6′(B)], 6.94 (2H, *d*, *J* = 8.5 Hz, H-3′(E), 5′(E)].

Afzelechin-(4α→8)-afzelechin octaacetate (**1a**)

Acetylation of **1** with acetic anhydride and pyridine gave octaacetate **1a**. Pale yellow gum. ^{1}H NMR ($CDCl_3$): δ 1.60, 1.90, 2.00, 2.25, 2.30, 2.35 (18H, each *s*, 6 × OAc), 2.27 (6H, *s*, 2 × OAc), 2.66 [1H, *dd*, *J* = 16.6, 8.1 Hz, H-4α(F)], 2.94 (1H, *dd*, *J* = 16.6, 5.9 Hz, H-4β(F)], 4.48 [1H, *d*, *J* = 9.5 Hz, H-4(C)], 4.76 [1H, *d*, *J* = 10.3 Hz, H-2(C)], 4.96 [1H, *d*, *J* = 8.3 Hz, H-2(F)], 5.06 [1H, *m*, H-3(F)], 5.66 [1H, *dt*, *J* = 2.2, 9.6 Hz, H-3(C)], 6.47 [1H, *d*, *J* = 2.2 Hz, H-6(A)], 6.48 [1H, *d*, *J* = 2.2 Hz, H-8(A)], 6.63 [1H, *s*, H-6(D)], 6.96 [2H, *d*, *J* = 8.5 Hz, H-2′(E), 6′(E)], 7.00 [2H, *d*, *J* = 8.5 Hz, H-3′(B), 5′(B)], 7.04 [2H, *d*, *J* = 8.5 Hz, H-3′(E), 5′(E)], 7.11 [2H, *d*, *J* = 8.5 Hz, H-2′(B), 6′(B)].

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